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Tumor-Suppressor Genes: Cardinal Factors in Inherited Predisposition to Human Cancers

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A predisposition to the development of certain specific and familial cancers is associated with the inheritance of a single mutated gene. In the best-characterized cases, this primary mutation is a loss of function mutation consistent with viability but resulting in neoplastic change consequent to the acquisition of a second somatic mutation at the same locus. Such genes are referred to as tumor-suppressor genes. Classical examples are the *Rb*-1 gene associated with the development of retinoblastoma and the *p53* gene, which is associated with a wider range of neoplasms, including breast cancer. Other tumor-suppressor genes have been isolated which are associated with Wilms' tumor, neurofibromatosis, and inherited and sporadic forms of colorectal cancer. Some of these genes appear to act as negative regulators of mitotic cycle genes, and others may have different properties. The nature of these genes is discussed, as is the evidence for the involvement of tumor-suppressor genes in other inherited, and sporadic, forms of cancer. Some recent data on the Wilms' tumor gene, *WT1*, and on the involvement of the *p53* gene in breast cancer are presented, and the importance of genomic imprinting in contributing to the excess of suppressor gene mutations in chromosomes of paternal origin is considered.

Introduction

Cancers are genetic diseases that are a consequence of alterations in the structure, expression, and hence function of usually more than one of a variety of genes. Transformation to a neoplastic state can be viewed as an untoward outcome of the competing forces of cellular differentiation, hibernation (quiescent stem cells), or programmed death (apoptosis) on the one hand, and proliferation on the other; the genes and genetic systems involved in controlling these processes are therefore obvious candidates in the quest for genetic factors of importance. In a normal proliferating tissue, the maintenance of homeostasis depends upon the retention of an appropriate population of stem cells with the propensity for proliferation and the production of the required number of differentiated cells for that tissue. Such a system depends upon a balance between controlling signals specifying proliferation and those inhibiting the passage of cells through a cell cycle. These signals, and the genes that specify them, may be viewed as positive or negative, promoting or inhibiting a programmed chain of events. In a somewhat oversimplified way, we consider those genes which, in an altered or overexpressed form, act in a positive and dominant way to promote proliferation as oncogenes and those in which mutational change, usually (but not always) expressed in a homozygous or hemizygous state, results in a negation of their normal inhibitory action on proliferation as tumor-suppressing genes.

The evidence for dominantly acting oncogenes stemmed initially from studies on virally induced tumors in rodents and chickens, and there are now at least 50 or so defined oncogenes in the human genome. The normal functions of these genes are to code for growth factors, growth factor receptors, second messenger proteins, or transcription factors as components that regulate normal cell growth and proliferation. In their altered, or overexpressed forms, they can, however, cooperate in inducing cell transformation *in vitro* or tumorigenesis *in vivo*. The importance of oncogenes in the initiation and development of human cancers is well established and has been reviewed extensively (*1,2*); such genes are therefore referred to only briefly here in the context of our discussion on tumor-suppressor genes. The idea that other genes in the genome act as tumor-suppressor genes stems from three kinds of observation: on cell hybrids between tumorigenic and nontumorigenic cells; on mutations, including constitutional loss of heterozygosity (LOH) at specific chromosomal sites, in a variety of inherited predispositions to cancer in childhood; and on acquired LOH at specific chromosomal sites in specific sporadic tumors. The early studies of Harris and colleagues (*3*) were among the first to clearly demonstrate that the property of tumorigenesis is lost in hybridomas between certain tumorigenic and nontumorigenic cells but is regained in descendant segregant cells that have lost specific chromosomes. This approach of introducing genomes, chromosomes, or genes from normal cells into their tumorigenic counterparts has

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been used extensively over the past 20 years as a means of identifying, or confirming the nature of, tumor-suppressor genes (*4*).

The mutations in oncogenes that convert them into transforming genes are often relatively specific and invariably result in an altered, but functional, gene product. Transformation may also follow as a consequence of inappropriate expression, either in time or in quantity, and the nature and dominance of many of the known changes would in most cases be inconsistent with normal development and viability of an early embryo/fetus. In contrast, those suppressor genes that are revealed by loss of function might be expected to yield a wider range of mutations many of which would be viable in the heterozygous state. Hence in a two-hit model of tumorigenesis which proposes that both copies of a tumor-suppressor gene be functionally inactivated for the development of a tumor, such genes in their heterozygous state could result in the inheritance of a predisposition to cancers. Most types of human cancer exist in both sporadic and inherited forms. Indeed, identification and characterization of the first tumor-suppressor gene followed from studies on the inherited childhood cancer of retinoblastoma. It may be instructive, therefore, to review briefly some of the developments in our understanding of the role of tumor-suppressor genes in the better understood inherited cancer predispositions, in which some of the relevant genes have been cloned and information is available on their function.

Retinoblastoma

Retinoblastoma, a tumor of the retina which affects some 1 in 5000 children, occurs in two forms: 30–40% of cases have bilateral and usually multifocal tumors that are evident at an earlier age than the commoner unilateral cancers. The earlier onset of multiple tumors is often associated with a familial history, and in 1971 Knudson (*5*) proposed that these tumors arise from two sequential mutations. In the familial form the first mutation is inherited and therefore constitutional and the second mutation is acquired and somatic; in the sporadic form, both mutations are somatic. A constitutional deletion of band q14.1 on chromosome 13 was reported in a number of familial cases (*6*), and similar, but somatic and tumor-specific, deletions in a number of sporadic tumors (*7*). The alteration was therefore considered to be a loss, or loss-of-function mutation at 13q14. Cavenee et al. (*8*), using a series of DNA markers for 13q, showed that the chromosome 13 from the nonaffected parent was lost in familial tumors and that the LOH thus acted to uncover an inherited constitutional mutation. Retinoblastoma appeared therefore to be a consequence of mutations affecting both alleles of a single gene (*rb*1) so that although the predisposition to this condition is inherited as an apparent autosomal dominant, at the cellular level the expression of neoplasia is a consequence of recessive changes.

The studies of Friend et al. (*9*) and Lee et al. (*10*) identified the gene responsible for retinoblastoma and showed that it codes for a 928 amino acid protein, p105-Rb. A number of studies have since reported a range of different mutations, including large and small deletions, and point mutations that alter amino acid sequence or splicing products, which yield an absent or inactive protein in tumor cells and cell lines (*11–13*). The Rb protein is a nuclear protein present in all types of cells and is found in phosphorylated and nonphosphorylated forms (*14*). The state of phosphorylation alters during the cell cycle, being maximal at the beginning of the DNA synthesis S phase and minimal after mitosis and entry into G1 (*15,16*). The stimulation of quiescent T lymphocytes to proceed through a mitotic cycle results in phosphorylation of p105-Rb (*17*) and the loss of proliferative capacity in senescent human fibroblast cultures exposed to growth factors is associated with the absence of phosphorylation. Overall the data would suggest that phosphorylation of Rb is necessary for cell cycling, and in particular for the transition from the G_1 to S phase, and that in its hypophosphorylated form the normal protein is associated with the suppression of mitosis. This suppression can be negated not only by phosphorylation, but also by inactivation of the Rb protein following its association with the tumorigenic proteins of a variety of DNA tumor viruses, i.e., adenovirus E1A, SV40 and polyoma virus large T, and human papillomavirus E7 (*18–21*). Some mutant Rb proteins cannot undergo phosphorylation and do not complex with viral tumorigenic proteins (*12*). *In vivo* the Rb protein complexes with a number of cellular proteins which interact with the same region that is important in binding DNA viral tumor proteins and the region frequently found mutated in tumors in which Rb is implicated (*22*). Recent studies have identified a specific binding of Rb proteins with the transcription factor E2F (*23–25*), a factor which also binds to the promoters of several cellular proliferation related genes, including the cell cycling protein cyclin A (*26*). The expression of such genes could therefore be regulated through Rb binding/sequestration of essential transcription factors.

The evidence that the *Rb*1 gene acts as a tumor-suppressor gene was strongly supported by the demonstration that the introduction of normal Rb into retinoblastoma or osteosarcoma cell lines devoid of normal p105-Rb suppresses the growth and tumorigenicity of the cells (*27*). *Rb* mutations are indeed found sporadically in a variety of human tumors, and suppression of tumorigenicity has also been reported following the introduction of a normal *Rb* gene into a human prostate cancer cell line (*28*) and into a bladder carcinoma cell line (*29*). Although, to date, there has been no report of a transgenic mouse containing a mutated *Rb* gene, Windle et al. (*30*) reported the occurrence of retinal tumors in transgenic animals in which the SV40 T antigen was specifically expressed in the retina. The evidence indicates that the T antigen protein in the retinoblastoma cells bound to p105-Rb, supporting the role of the inactivated Rb protein in retinal oncogenesis.

Although retinoblastoma is the paradigmatic example of the consequences of a null mutation at a tumor-suppressor locus, it remains unclear whether additional mutational steps are necessary for the full emergence of tumors. It has been remarked that nonmalignant retinomas can occur in *Rb* carriers (*31*), but there is no information on

their frequency or on their genetic constitution. There is considerable evidence that malignant retinoblastomas may contain a range of consistent chromosomal abnormalities, including abnormalities of 1q and 17p, in addition to the mutation at 13q14 (*32,33*).

Wilms' Tumor

Wilms' tumor, a nephroblastoma with an incidence of around 1 in 10,000, is one of the most common solid tumors of childhood. Most cases are sporadic and unilateral, but a small proportion (~8%) are bilateral, and a further 1% are clearly familial. From a study of the age at onset of unilateral and bilateral cases, Knudson and Strong (*34*) suggested that the etiology of Wilms' tumor parallelled that of retinoblastoma and that the occurrence of Wilms' tumor involved two mutational events at a single gene locus.

Cytogenetic studies of sporadic tumors and of blood lymphocytes and tumors in inherited forms of the disease initially implicated a gene at chromosomal band 11p13 in the etiology of some, but not all, cases of Wilms' tumor (*35*). This suggestion was supported by the results of molecular genetic studies using relevant DNA markers (*36–38*), and the data were consistent with the notion that a mutation of one 11p13 allele and subsequent mutation or loss of the homologous allele were necessary, but perhaps not sufficient, for tumorigenesis. Recent results of chromosome walking and jumping to detect CpG island sites in this region have led to the independent isolation of cDNA clones that encode a transcription factor with four zinc fingers and a proline-rich domain; there is strong evidence that it is a candidate *WT* gene (*39,40*). Work in our own unit (*41*) on the expression of this gene in the early human fetus shows that it is expressed in fetal kidney, gonads, and spleen, as might have been expected if the gene was indeed the *WT*1 Wilms' tumor gene. Conclusive evidence that this is so was recently presented by Huff et al. (*42*), who detected a constitutional heterozygosity for a small intragenic deletion in *WT*1 in a patient with bilateral Wilms' tumor and then indicated that homozygosity for this deletion was present in both tumors. Loss of constitutional heterozygosity for linked DNA markers showed that the homozygosity in the bilateral tumors had been engineered by two different genetic events. Work in our unit (*43*) has resulted in identification of two different structurally critical zinc-finger point mutations in two Wilms' tumor patients; in the patient with bilateral tumors, the mutation was constitutional and present in a hemizygous state in one tumor. These data fully support the notion that the *WT*1 gene is a tumor-suppressor gene.

Since this gene codes for a transcription factor, the identification of target sequences to which it binds is important. Rauscher et al. (*44*) have expressed the zinc-finger domains of the *WT*1 gene in *Escherichia coli* and used this protein as an affinity matrix for the isolation of DNA target sequences. A sequence was identified that turns out to be closely similar to a binding sequence recognized by the product of the early growth response gene-1, *EGR*-1, a zinc-finger protein that is induced by mitogenic stimuli. A deletion in the third zinc finger of the *WT*1 protein, a mutation that occurs naturally in Wilms' tumor, was shown to reduce the binding activity severely. The possibility exists that the normal *WT*1 protein may suppress the binding of proteins coded for by the *EGR*-1 gene family, including genes like *fos* and *jun* which respond to mitotic stimuli and regulate gene transcription.

Although the two-hit tumor-suppressor model would appear to be applicable for certain cases of Wilms' tumor the picture is not as simple as that seen in retinoblastoma. Studies of two large families with Wilms' tumor have shown that the predisposition is not linked to 11p, implying the importance of a mutation elsewhere in the genome (*45,46*). Moreover, there is evidence for a second *WT* locus at 11p15 (*47,48*). Wilms' tumor is observed in patients with the Beckwith-Weidemann syndrome and the gene for this syndrome is tightly linked to the insulin and insulinlike growth factor-2 genes at 11p15 (*49*), and there is evidence for imprinting at the Igf2 locus (see below). At the present time, data on alterations to *WT*1 genes are insufficient to determine if they provide, in addition to classic tumor-suppressor activity, a dominant/negative heterozygous gene effect (*cf* p53) or, indeed, whether additional mutations elsewhere in the genome are necessary.

Von Recklinghausen's Neurofibromatosis

Von Recklinghausen's neurofibromatosis is a common autosomal dominant disorder involving tissues derived from the neural crest, and in particular the peripheral nervous system, where it is associated with the development of café-au-lait spots and benign neurofibromas in over 90% of cases. The condition has a prevalence of around 0.2–0.3 per 1000 individuals, and among its more serious complications are the occasional development of neurofibrosarcomas and optic gliomas. All cases result from the inheritance of a mutant allele; since the mutation rate of this rather large gene (*NF*-1) is high, approximately 1×10^{-4} (*50,51*), then 40–50% of all cases must involve new mutations.

The *NF*-1 gene was mapped, by linkage analysis, to 17q (*52,53*) and a candidate gene isolated from Von Recklinghausen's neurofibromatosis patients with constitutional translocations affecting 17q11.2. This gene had undergone a variety of different mutations in various patients, implying that its loss of function was a causative factor in the syndrome (*54–56*). The gene is large, encoding a 13-kb transcript and a protein of at least 2485 amino acids; it is expressed in most, if not all, tissues and is transcribed in human/rodent hybrids containing a normal chromosome 17 but not in hybrids containing balanced translocations from Von Recklinghausen's neurofibromatosis patients (*56*). The findings are therefore consistent with the idea that the gene acts as a tumor-suppressor gene, but it is not known whether second-hit mutations in the normal copy of *NF*-1 are necessary for the development of the neurofibrosarcomas, or whether mutations at other sites may be required.

The mode of action of the *NF*-1 gene in its tumor-suppressing role is unclear, but there is abundant evidence that it plays a role in signal transduction pathways. A series of studies (*57-60*) have shown that the gene encodes a cytoplasmic protein with a large region of similar amino acid sequence and functional homology to mammalian GAP and to the IRA1 and IRA2 gene products of *Saccharomyces cerevisiae*, which are known to inhibit yeast *ras*. The *NF*-1 protein has been shown to interact with mammalian p21*ras*, and indeed a segment of human *NF*-1 cDNA can inhibit the action of both wild-type and mutant H-*ras* genes in yeast.

Familial and Sporadic Colorectal Cancers

The classic syndrome of familial colorectal cancer is that associated with dominant autosomally inherited familial adenomatous polyposis (FAP), although there are other nonpolyposis familial forms. FAP has a prevalence of around 1 in 10,000, whereas various lines of evidence would suggest that other major genes may be responsible for a significant proportion of colorectal cancers (*61,62*), and familial studies show a 3-fold increased risk in first-degree relatives of colorectal cancer probands.

FAP is characterized by the development of hundreds, or carpets of thousands, of colorectal adenomas, some of which develop into frank carcinomas, and is transmitted by a gene (*APC*) mapping to the q21 region of chromosome 5 (*63–66*). Adenomas in FAP patients show no loss of the *APC* region in their mildly or moderately dysplastic form, but those that develop into carcinomas appear to be associated with a deletion of the *APC* region in the normal chromosome transmitted from the unaffected parent (*67*). These findings suggest that the *APC* gene acts as a cell recessive tumor-suppressor gene, a conclusion reinforced by the demonstrations that transfected chromosomal fragments that include the 5q21 region reverse the neoplastic phenotype of cultured rodent fibroblasts (*68*) and that the introduction of a normal chromosome 5 into human colorectal epithelial cells completely abrogates their neoplasticity (*69*).

The development of colorectal carcinomas involves a number of genetic changes, and, although *APC* loss/inactivation may be a necessary event, it may not be entirely sufficient for the development of overt neoplasticity. Insight into other important mutational events has stemmed from a variety of studies on sporadic colorectal tumors. Of special interest are the observations of a number of authors who report heterozygous loss of the *APC* region in up to more than 50% of sporadic tumors studied (*70–72*). Vogelstein and colleagues in particular have demonstrated a high frequency of heterozygous loss and of mutation within one known tumor-suppressor gene, p53 on 17p13.1 (*73*), and one probable tumor-suppressor gene, *DCC* on 18q (*71*). A candidate *APC* gene has recently been isolated which is expressed in normal human and rodent colorectal mucosa and a variety of other tissues (*74*). The gene, located in the *APC* region, was observed to be mutated in three colorectal carcinomas and is referred to as the *MCC* gene (mutated in colon cancer). *MCC* encodes an 829-amino-acid protein with a short region of similarity to a G protein (*cf NF*-1); in the absence of confirmation of a constitutional mutation in the *MCC* gene in FAP patients, however, the question of whether the *MCC* gene is indeed the *APC* gene is still open.

The *DCC* gene on chromosome 18q, a region that shows heterozygous loss in more than 80% of colorectal carcinomas, was identified by Fearon et al. (*75*) and shown to be expressed in most normal tissues but with a very greatly reduced or absent expression in colorectal carcinomas. Mutations were detected in the gene in 12 of 94 carcinomas, and the predicted amino acid sequence shown to be closely similar to neural cell adhesion molecules and other related cell surface glycoproteins.

The *p53* gene on chromosome 17p is perhaps the most studied of the known tumor-suppressor genes and is implicated not only in colorectal tumors but in a wide range of other cancers (Table 1). The p53 protein is a 393-amino-acid nuclear phosphoprotein which is expressed at very low levels and with a short half-life in the majority of normal mammalian cells (*90*). It was originally discovered by coprecipitation with the transforming protein, large T, in SV40-infected cells (*91*) and later shown to form specific complexes with the transforming proteins of other oncogenic DNA viruses: E1B of adenovirus (*92*) and E6 of HPV-16 and -18 (*18,93*). The abundance and state of phosphorylation of p53 is cell cycle-dependent, being minimal immediately following mitosis and increasing markedly in cells in the S and G_2 phases (*94*). The protein acts as a substrate for cdc2, a predominantly nuclear protein kinase that regulates the commitment of cells to undergo DNA synthesis and which, in a dephosphorylated form, triggers cells to enter mitosis (*95,96*). These findings parallel remarkably closely those observed with the Rb protein, suggesting a functional connection between p53 and Rb-105, with the implication that both are involved in a common, negatively regulating pathway in the proliferative cycle. The functional similarity between p53 and Rb is further reinforced by the finding in herpes virus-infected cells that these proteins, together with other host replication proteins, colocate at nuclear sites of viral replication (*97*).

Initially, p53 was considered to be an oncoprotein, but cDNA clones of *p53* that had transforming properties were later shown to be mutant (*98–100*). Wild-type *p53*, but not mutant forms, suppress the transforming properties of other oncogenes as well as the growth of transformed cells in culture (*101*). The tumor-suppressing role of *p53* has been supported by two recent studies: first, by Chen et al. (*102*), who showed that the introduction of wild-type *p53* genes into human osteosarcoma cells lacking endogenous p53 completely abrogated the neoplasticity of the cells, whereas the introduction of mutant *p53* conferred a limited growth advantage; second by Baker et al. (*103*), who showed that transfection of the wild-type *p53* gene *in vitro* resulted in a suppression of growth of colorectal carcinoma but not adenoma cells. We should not lose sight of the fact that none of the different mutant *p53*s assayed, by transformation *in vitro* possesses the suppressing activity of the normal gene (*104–106*).

Table 1. Loss of heterozygosity (LOH) at 17p and *p53* mutations in various human tumors.

Tumor site	No. of tumors or cell lines[a]	No. with LOH 17p (%)	No. with *p53* mutation (%)	References
Bladder	18	17 (94)	11 (61)	Sidransky et al. (*76*)
Brain	4	4 (100)	2 (50)	Nigro et al. (*77*)
Breast	60	33 (55)	15 (25)[b]	Prosser et al. (*78*, unpublished data)
	11[a]	–	11 (100)	Bartek et al. (*79*)
	32[c]	32[a]	11 (29)	Borreson et al. (*80*)
	52	27	2 (13)	Chen et al. (*81*)
Colorectum	10[a]	–	6 (60)	Rodrigues et al. (*82*)
	26+4[a]	0	5 (17)	Baker et al. (*73*)
	20+8[a]	28	24 (86)	Baker et al. (*83*)
Liver cells	16	–	8 (50)	Hsu et al. (*84*)
	10	3 (60)	5 (50)	Bressac et al. (*85*)
Lung	30[a]	–	17 (57)	Takahashi et al. (*86*)
	40	–	28 (70)	Iggo et al. (*87*)
Esophagus	14+4[a]	–	7 (39)	Hollstein et al. (*88*)
Ovary	16	11 (91)	11 (69)	Eccles et al. (*89*)

[a]Cell lines.
[b]Minimum number; estimated frequency, > 40%.
[c]Selected for LOH 17p.

A study of *p53* in 58 colorectal tumors (*83*) showed that most tumors (~90%) with a loss of one 17p had a *p53* mutation on the remaining allele, but such mutations were less frequent (30%) in tumors containing both copies of 17p and were relatively rare in adenomas. This pattern indicates that the mutations and allelic losses become evident at around the time of transition from benign to malignant growth. The data also imply that the presence of a single mutant *p53* allele may exert a dominant-negative effect perhaps by binding to the wild-type protein and creating an inactive oligomeric complex; this implication is supported by a number of studies (e.g., *104,107*). It is worth noting that if the tumorigenic effects of *p53* are dependent on knocking out gene activity, few gross deletions and rearrangements have been observed. Much more frequent are point mutations in conserved regions, resulting in alterations in amino acid sequence, which appear to promote proliferation and confer some selective advantage to the tumor cells (*100*).

Familial and Sporadic Breast and Ovarian Cancers

Familial forms of breast cancer have been recognized for well over a century, and a woman's risk of developing the disease over a given period is known to be increased by up to 3-fold if a first-degree relative has had breast cancer and by 5- to 10-fold if that relative had bilateral disease. Various studies point to the importance of inherited factors, and in some cases the evidence would imply the presence of an autosomal dominantly inherited susceptibility allele (*108*). A number of groups have used polymorphic DNA markers in linkage studies to localize a predisposing gene, and our own studies produced suggestive evidence of linkage in families with early, premenopausal and usually bilateral, cancer to a region on chromosome 17p. None of these studies, however, including our own, yielded conclusive evidence probably because of the heterogeneity of the disease. Convincing evidence was recently published by Hall et al. (*109*), who obtained a log of the odds score of +5.98 for linkage of breast cancer susceptibility to the marker D17S74 in early-onset families and negative scores in late-onset families. We confirmed these findings in some, but not all (*108*), of cancer families we studied so that a predisposing gene is located in chromosome 17q21 in some families. Although there are various possible candidate genes within that chromosomal region, the particular susceptibility gene has not been identified.

Studies of somatic chromosomal changes in breast cancer tissues, and particularly those revealing consistent LOH, have identified a number of sites of possible tumor-suppressor genes. Our early studies (*110*) showed that more than 60% of sporadic breast cancers from a consecutive series of patients had LOH for a region of chromosome 17p; these results have been extended by ourselves (*111*) and confirmed by others (*112,113*). We further showed that in 50% of the tumors there was overexpression of p53 mRNA and that this was correlated with LOH of 17p. Overexpression of p53 is associated with mutant forms of the gene which result in a more stable gene product (*99,100*). Detailed genomic DNA sequence analysis of exons 5-9 (*78*, unpublished data)—which include most of the conserved regions in which the majority of *p53* mutations are found in other cancers—revealed that *p53* mutations were present in at least 25% (up to an estimated 40%) of the 60 breast tumors studied. The study of Varley et al. (*113*) extended these findings: LOH at 17p13 and/or expression of mutant *p53* was seen in 86% of 74 breast cancers, underlining the fact that alterations involving *p53*, either by loss of one allele and/or intragenic mutation, are by far the most common genetic change in primary human breast tumors.

Our suggestive linkage data on early familial breast cancer and markers on 17p (log of the odds score of +2.0),

and the finding of a high frequency of mutations in the *p53* gene at 17p13.1 in sporadic breast cancers, led us to study the gene in normal blood cell DNA from individuals in five families with breast cancer. No constitutional mutation of *p53* was found (*114*). Recently, however, Malkin et al. (*115*) and Srivastava et al. (*116*) described the presence of constitutional *p53* mutations in family members with Li-Fraumeni syndrome. This syndrome is a rare autosomal, dominantly inherited condition that is characterized by a diverse range of neoplasms, including breast cancers and soft-tissue sarcomas, which develop relatively early in life. The reported studies on DNA from normal skin fibroblasts or lymphocytes from affected and unaffected members of six different families describe the presence of a constitutional point mutation (a base substitution) in the *p53* gene at codon 248 in three families, at 245 in two, and at 258 in one. A linkage study on one family confirmed the cosegregation of the mutated chromosome with the occurrence of neoplasms. The most frequent neoplasm observed was breast cancer (60 of a total of 231 primary cancers), which was about twice as frequent as soft-tissue sarcomas or brain tumors. All individuals appeared to have a single wild-type *p53* allele, and were therefore constitutional heterozygotes for the mutation, and at least one parent and two grandparents carried a *p53* constitutional mutation but had not developed cancer. These findings are rather dramatic, but it should be noted that we have observed no mutation in one Li-Fraumeni family studied in our laboratory and that as yet unpublished data from other laboratories describe such families both with and without *p53* mutations. The finding that individuals who are heterozygous for a constitutional mutation in the evolutionary conserved region III of the *p53* gene (*117*) are viable and prone to the development of a variety of early cancers is a signal discovery, and the fact that some gene carriers do not appear to manifest neoplastic disease begs the question of how frequent this kind of mutation is in the general population, and whether it is associated with inherited cancer predisposition in non-Li-Fraumeni families.

Answers to these questions should be forthcoming shortly, but meanwhile it is relevant to note that the introduction of constructs of mutated *p53* genes into normal mouse embryos results in the development of neoplasms in some 20% of the resultant transgenic animals (*118*). Although the transgenes were widely expressed most of the tumors seen were lung adenomas, osteosarcomas, and lymphomas, and the average age of incidence was 11 months. This long latent period and the absence of a correlation between levels of tissue expression of *p53* and the occurrence of tumors, imply that deregulation of *p53* alone is insufficient for tumor formation and that other genetic, and perhaps tissue-specific epigenetic, changes may be involved.

Familial breast cancer is often associated with familial ovarian cancer, and a number of reports indicate a possible autosomal dominant mode of inheritance of ovarian cancer. Following the report of Hall et al. (*109*) showing linkage of early breast cancer cases in some families to a region on 17q, Narod et al. (*119*) confirmed this finding and, further, showed linkage between familial ovarian cancer and the same chromosome region, i.e., 17q12-23. In parallel with our studies on breast tumors, we have noted the involvement of 17p in sporadic ovarian tumors; 13 of 16 advanced ovarian serous adenomas showed LOH for 17p, and 11 of these tumors had a *p53* mutation (*89*). In these tumors LOH at 17p, detected by markers closely linked to p53, correlates closely with the presence of a *p53* mutation in the remaining homolog.

Loss and mutation at the *p53* locus are clearly important features of both breast and ovarian cancers, but it should be noted that there is some evidence in breast cancer for the involvement of a second locus on 17p some 20 Mb telomeric to *p53* (*81,120*). Moreover, studies of sporadic breast tumors have uncovered high frequencies of LOH at other chromosomal sites, e.g. 1p, 1q, 11p, 18q (*121–123*); and loss of the *Rb* gene has also been reported in cases of ductal breast cancer (*14,124*). The genes that may be involved at these other sites have not been identified, but tumor-suppressor genes other than those on 17p and 17q may be important in breast cancer.

Other Inherited Cancer Predispositions and Suppressor Genes in Other Sporadic Cancers

In addition to the genes involved in the inherited cancer predispositions and in sporadic cancers already referred to, loci involved in other inherited cancers have been assigned to specific chromosomes (Table 2). Although these cancer-associated syndromes appear to be inherited as dominant autosomal conditions, the genes involved might represent classical recessive tumor-suppressor genes; but none has as yet been characterized. It is unlikely to be merely coincidental that there is a chromosome 3 locus involved in the inherited von Hippel-Lindau syndrome with its associated renal carcinomas, a similar locus involved in a translocation of chromosome 3 that segregates with renal cancer in one large family (*133*), and a LOH for this chromosome region described by Kovacs et al. (*134*) in 18 out of 21 sporadic renal cancers. A consistent loss of 3p has also been noted in sporadic lung cancers (*135,136*). A candidate tumor-suppressor gene (a receptor protein-tyrosine phosphatase) at the 3p21 region has indeed recently been isolated (*137*). In a number of renal carcinoma cell lines and lung tumor samples, one allele of this gene was lost, but there is no conclusive evidence of its tumor-suppressing role in these cancers. Similarly, the consistent involvement of chromosome 22 in sporadic meningiomas (*138*) and the assignment of the gene for neurofibromatosis type 2 associated with acoustic neuromas to this chromosome should also be noted.

None of the conditions listed in Table 2 appears to be associated with inherited defects in the repair of mutational damage, but genes involved in one form of xeroderma pigmentosum, Cockayne's syndrome (*139*), and Bloom's syndrome (*140*)—three recessive autosomal conditions involving defective DNA repair—have recently been identified. The gene defects in these three, and in other putative DNA repair deficiencies, are presumably associated with inherited cancer predisposition by virtue of

Table 2. Examples of dominantly inherited cancer predispositions in which the mutated gene has not yet been identified.

Syndrome	Tumor	Chromosome assignment	LOH of assigned chromosome in tumors	References
Multiple endocrine neoplasia type 1	Pituitary adenomas/pancreas	11q (centric)	+	Larson et al. (*125*)
Multiple endocrine neoplasia type 2	Medullary carcinoma of thyroid	10	–	Mathew et al. (*126*) Landsvater et al. (*127*) Nelkin et al. (*128*)
Neurofibromatosis type 2	Acoustic bilateral neuromas	22	+	Seizinger et al. (*129*), Rouleau et al. (*130*)
von Hippel Lindau	Renal-cell carcinoma, CNS, hemangioma, pancreas	3p	+	Seizinger et al. (*131*)
Dysplastic nevus familial melanoma	Melanoma	?	?	Bergman et al. (*132*)

LOH, loss of heterozygosity.

inherited genomic instability and heightened mutation sensitivity. These genes may not be tumor-suppressor genes in the classical sense.

Genomic Imprinting and Patterns of Inheritance and Expression of Mutated Suppressor Genes

It is evident from studies on tumor-suppressor genes in various inherited cancer predispositions that a principal mechanism in tumorigenesis is the unmasking of an initial (inherited) mutation (and in some cases its later duplication via disomy) by a subsequent mutation that eliminates the wild-type allele. This elimination may involve whole or partial chromosomal loss (*8,72*). Such unmasking can be recognized by loss of heterozygote status in appropriate cases, and the parental origin of the original mutated chromosome can be determined. A similar analysis may be undertaken in nonfamilial cases in which a new germ-line mutation has occurred and in comparable sporadic tumors. This type of analysis has been performed on a range of tumors and has disclosed a marked distortion in the parental origin of the initially mutated chromosome (Table 3). Various mechanisms have been proposed to account for the observed preponderance of mutation in the paternally derived chromosome, and the consequent loss of the maternal allele, with much of the discussion centering upon the importance of the phenomenon of "genomic imprinting."

"Genomic imprinting" is the term used to refer to the differential expression (transcriptional inactivation) of whole haploid chromosome sets in some insects (*150*) or of segments of autosomes in mice (*151,152*), which is dependent upon the sex of the parent from which they are inherited. Studies in the mice suggest that such parental imprinting, which suppresses the expression of segments of the genome, is a consequence of methylation (*153*). The methylation pattern is erased in primordial germ cells and then reintroduced, in a modified form, in later germ-cell stages or in early embryogenesis, the introduced pattern being established by the parental sex of the germ cells (*154*). The first clear evidence that the phenomenon of genomic imprinting occurs in humans followed from the discovery that children with Prader-Willi syndrome have a constitutional deletion of chromosome 15q11-13 which was inherited from the father (*155*), whereas children with the very different Angelman syndrome may have the very same deletion but inherited from the mother (*156*). In some Prader-Willi patients in whom the deletion is not evident

Table 3. Parental origin of mutated allele in tumors (individuals) in some inherited cancer predispositions.

Tumor/syndrome/ allele (chromosome)	No. of families/cases	Origin of related/mutant allele in tumor[a]		References
		Paternal	Maternal	
Retinoblastoma *Rb* (13q1.4)	22 (constitutional)	20	2	Ejima et al. (*141–143*) Dryja et al. (*142*) Zhu et al. (*143*)
Osteosarcoma *Rb* (13q14)	13 (sporadic)	12	1	Toguchida et al. (*144*)
Neurofibromatosis[a] *NF1* (17q11.2)	14 (constitutional)	12	2	Jadayel et al. (*145*)
Wilms' tumor *WT* (11p13)	18 (sporadic)	16	2	Schroeder et al. (*146–148*), Mannens et al. (*147*), Huff et al. (*148*)
Rhabdomyosarcoma	6 (constitutional)	6	0	Serable et al. (*149*)

[a]Parental origin of mutation by pedigree linkage analysis and not tumor biopsy.

there is no paternal contribution of relevant loci and the patients are disomic for maternally derived chromosome 15s.

Genetic (mutations) or epigenetic (imprinting) inactivation of a tumor-suppressor gene is functionally equivalent, and it is obvious that imprinting may be an important factor in carcinogenesis. Various hypotheses have been proposed to account for the large excess of mutations arising in suppressor genes in paternal germ cells in inherited cancers and in somatic cells in sporadic cases, and for the preferential loss of maternally inherited suppressor loci. Many of these include proposals that imprinting is associated with a high mutation rate (*145*), that mutations may affect the genes that control imprinting (*149*), and that imprinted chromosomes (or segments) may be more subject to loss. Relevant to these suggestions are two recent reports. The first is by Sakai et al (*157*) on the methylation pattern at the 5′ end of the retinoblastoma gene, including its promoter region and the first exon, in the DNA from 56 primary retinoblastomas. It transpired that both copies of the *Rb*1 gene in four of these tumors from patients with unilateral retinoblastoma were hypermethylated, whereas the gene in their blood cells was not. No mutation was observed, implying an epigenetic origin for the loss of tumor suppressing activity. The second study was by Henry et al. (*158*) on the etiology of the Beckwith–Weidemann syndrome, which is a fetal overgrowth syndrome that arises sporadically, or as a result of the inheritance of an autosomal dominant mutation linked to 11p15.5, and is often associated with Wilms' tumor. A substantial proportion of BWS cases, of both inherited and sporadic forms, have now been shown to be associated with paternal disomy/maternal nullisomy of the 11p15 region (*158*). Evidence from studies in mice shows that the insulin-like growth factor-2 gene, which is located at the 11p15.5 region in the human genome, is imprinted and is not expressed on the maternally derived chromosomes (*159,160*). These findings lend support to the implication that there may be a tumor suppressor gene at this site in the human genome which is not expressed in maternally derived chromosomes. It is also relevant to note that the short arm of chromosome 11 is a hot spot for hypermethylation in various human neoplasms (*161*), and there is direct evidence of methylation of a CpG island associated with the *WT*1 gene in some Wilms' tumors (*162*).

Concluding Comments

The emergence of metastatic cancer undoubtedly depends upon mutational or epigenetic changes in a number of genes. The order in which these changes occur may be less important than the number and types of changes (*163*). The genes involved may act in a positive fashion as oncogenes promoting proliferation or in a negative fashion as suppressor genes whose function is to inhibit uncontrolled growth in some instances perhaps by promoting cellular differentiation or apoptosis. There must therefore be a wide variety of suppressor genes, and we are some way from a clear understanding of the modes of action of even the best known. Recent progress in this area has, however, been quite dramatic.

Genetic and molecular studies on lower eukaryotes, which have been extended to include mammalian cells (*164*), have identified families of molecules, cyclins, that undergo cell cycle associated fluctuations which reach their maximal levels at the G_2:M transition and are destroyed during mitosis. Some members of the families of cyclins (B type) interact with $p34^{cdc2}$, a kinase which is activated in cells entering mitosis and shows maximal activity at metaphase. Others (G_1 and A types), at least one of which may act as an oncogene (*165*), may be involved in the transition of cells into and through G1, and in the process of triggering cells to enter the S phase (*166,167*). The emerging understanding of these positive controls of cellular proliferation has thrown light on the negative role played by the wild-type Rb protein. As already indicated, the non-phosphorylated form of Rb blocks the entry of cells into S, a blockage that is removed by phosphorylation. Rb protein in these G1 cells has been found to be bound to the transcription factor E2F. This Rb/E2F complex disappears at the G1:S boundary following phosphorylation of Rb and is replaced by an E2F/cyclin A complex. Negative control of Rb would therefore appear to be exerted by preventing interaction of the transcription factor with cyclin promoters. Two groups have recently provided further support for this role (168,169), by demonstrating that mutant forms of the Rb protein are no longer able to bind to E2F.

What of the roles of tumor-suppressor genes that act dominantly in negating the effects of oncogenes or in promoting cellular differentiation and apoptosis? Genes such as the K-*rev*-1 *ras*-related gene have been shown to have dominant suppressor activity against a specific set of oncogenes (*170*), but no gene of this type has been associated with inherited cancer susceptibility. An increasing number of differentiation pathways have been identified, particularly in relation to the hematopoietic system, and a number of important genes identified, but none has been implicated in inherited cancer predispositions. A large

Table 4. Human genes that may have tumor-suppressor activity.

Gene		Gene product and function
*Rb*1	Nuclear phosphoprotein	Modifier of transcription factor that regulates mitotic cycle genes
p53	Nuclear phosphoprotein	Like *Rb*1, may regulate mitotic cycle genes
*WT*1	DNA binding zinc finger protein	Transcription factor
DCC	Sequence similar to outer cell surface glycoprotein	Cell adhesion and cell–cell interaction
MCC	G-protein activator?	Signal transduction
	Coiled-coil structural protein	Cell structure
*NF*1	Cytoplasmic GAP-like protein	Intracellular signal transduction
PTPG	Transmembrane protein–tyrosine phosphatase γ	Dephosphorylation of tyrosine
K-rev 1	Inner cell surface associated G-protein	Interferes in the interaction between *ras* and its effector

number of genes are involved in normal growth and differentiation but it does not follow that all of these will possess the properties of tumor-suppressor genes. It may be relevant to note the increasing interest in the genes that are responsible for programming cell death or in blocking such programs, e.g., the *bcl*2 oncogene (*171*), and the recent report that wild-type p53 induces apoptosis in myeloid leukemic cells which is inhibited by the cytokine interleukin-6, which itself promotes monocyte differentiation (*172*).

Finally, it is evident that the term "tumor-suppressor genes" is applicable to a wide variety of genes involved in normal cellular functioning. The unifying characteristic appears to be the necessity of having one functioning wild-type allele in order to prevent abnormal proliferation or differentiation at particular stages of cell growth. It is this feature that permits normal activity in a hemizygous state and underlies the association of some of these genes with certain inherited cancer predispositions. To date the genes identified within this category are of diverse function (Table 4). Some may act within a narrow range of cell types and consequently be involved in the etiology of relatively few tumor types, as with WT1. Others, of fundamental importance to the growth and maintenance of a broad range of cells, will be found to be implicated in many tumors as with *p53* and *Rb*1. Since many of these genes appear to act as negative regulators of cellular proliferation, and their presence in a single copy is sufficient for the normal control of proliferation, they may well offer an important approach for the future therapeutic control of abnormal growth.

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